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Ethanol Leaf Extract of *Baphia longipedicellata* Ameliorates Metronidazole-Induced Oxidative Stress and Cytoarchitectural Alterations in the Hypothalamic-Pituitary Axis of Male Wistar Rats

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ABSTRACT
The hypothalamic–pituitary axis regulates reproduction, and its oxidative-stress vulnerability is key to reproductive toxicity from drugs. Metronidazole (MTZ) has been associated with adverse effects on this axis, potentially impairing male fertility. This study investigated the protective effects of ethanol leaf extract of *Baphia longipedicellata* (ELEBL) against metronidazole-induced oxidative stress and cytoarchitectural alterations in the hypothalamic-pituitary axis of male Wistar rats. Twenty-five adult male Wistar rats (190-210g) were divided into five groups (n=5). Group A received distilled water (10 mL/kg). Group B received MTZ (500 mg/kg) for 14 days. Groups C and D received MTZ followed by ELEBL at 200 mg/kg and 400 mg/kg respectively for 54 days. Group E received MTZ with a 54-day withdrawal period. After treatment, hypothalamic-pituitary tissues were harvested and evaluated for oxidative stress biomarkers (superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA). Histoarchitecture was assessed using Hematoxylin and Eosin (H&E) and Cresyl Fast Violet staining. MTZ administration significantly depleted SOD, CAT, and GSH while elevating MDA levels compared with controls (p<0.0001). ELEBL at both doses significantly restored antioxidant enzyme activities and reduced MDA to near-control values (p<0.05), with the 400 mg/kg dose demonstrating superior efficacy. Histological examination revealed that MTZ caused severe neuronal degeneration, vacuolation and pyknosis in the hypothalamus, along with marked degeneration of acidophils and basophils in the pituitary gland. ELEBL-treated groups showed near-normal architecture with preserved Nissl substance and well-distributed pituitary cell populations. These findings demonstrate that ELEBL attenuates metronidazole-induced oxidative stress and preserves hypothalamic-pituitary cytoarchitecture.

Keywords: *Baphia Longipedicellata*, Metronidazole, Hypothalamic-Pituitary Axis, Oxidative Stress

INTRODUCTION

The hypothalamic-pituitary axis constitutes the central regulatory machinery of the neuroendocrine system, integrating neural and hormonal signals to govern reproductive function (Clavijo & Hsiao, 2018). Within this axis, the hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins subsequently act on the testes to promote spermatogenesis and testosterone production (Odetayo *et al*., 2024; O'Donnell and Smith, 2026). The structural integrity of the hypothalamic-pituitary axis is therefore indispensable for male fertility and any disruption of its cellular architecture or function can precipitate reproductive failure (Sharma *et al*., 2022; Ma *et al*., 2025).

Oxidative stress represents a principal mechanism through which exogenous agents compromise neuroendocrine function (Qin *et al*., 2026). The hypothalamic-pituitary region is particularly susceptible to oxidative damage owing to its high metabolic rate, abundant polyunsaturated fatty acid content, and relatively limited antioxidant reserve compared with other brain regions (Dash *et al*., 2025). When reactive oxygen species (ROS) are generated in excess of the endogenous antioxidant capacity, they attack cellular macromolecules, including membrane lipids, proteins, and nucleic acids, culminating in neuronal injury and endocrine dysfunction (Garcia-Llorens *et al*., 2025). The antioxidant defense system of the hypothalamic-pituitary axis comprises enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and non-enzymatic antioxidants like glutathione (GSH), which function synergistically to neutralize ROS and maintain redox homeostasis (Jomova *et al*., 2024).

The vulnerability of hypothalamic neurons to oxidative stress is particularly consequential because these cells regulate the pulsatile secretion of GnRH, which is essential for downstream gonadotropin release (Şişli *et al*., 2022). Oxidative damage to hypothalamic neurons can disrupt GnRH pulsatility, leading to impaired LH and FSH secretion from the pituitary gonadotrophs (Roepke & Sadlier, 2021). Similarly, oxidative injury to pituitary cells, particularly the gonadotrophs (basophils), can directly compromise gonadotropin production and secretion (Nadia *et al*., 2026). The integrity of Nissl substance, which reflects the rough endoplasmic reticulum and protein synthesis capacity of

neurons, serves as a sensitive indicator of neuronal health and function (Bhati *et al*., 2023). Thus, agents that induce oxidative stress in the hypothalamic-pituitary axis can produce both structural and functional reproductive deficits.

Metronidazole (MTZ) is a nitroimidazole antibiotic extensively prescribed for infections caused by anaerobic bacteria and certain protozoa (Weir & Le, 2023). Its therapeutic efficacy notwithstanding, MTZ has been associated with adverse effects on the male reproductive system, including reduced sperm quality and impaired fertility (Azouz *et al*., 2026). The toxicity of MTZ is attributed to its reductive activation within cells, which generates ROS and nitro radical anions that overwhelm cellular antioxidant defenses (Yeh *et al*., 2026). Studies have demonstrated that MTZ exposure reduces sperm count and motility while increasing the proportion of morphologically abnormal sperm, effects that are mediated by oxidative damage to reproductive tissues (Kumari & Singh, 2013; Kong *et al*., 2025). Furthermore, MTZ can disrupt the hypothalamic-pituitary-gonadal (HPG) axis, leading to hormonal imbalances that impair spermatogenesis (Azouz *et al*., 2026).

Baphia longipedicellata Brumitt, a leguminous plant of the Fabaceae family, is indigenous to West and Central Africa and has a long history of traditional use in managing inflammatory conditions, diabetes, and male infertility (Oladoja *et al*., 2024). Phytochemical investigations have identified the presence of flavonoids, alkaloids, saponins, tannins, and terpenoids in the leaves, compounds that are well-recognized for their antioxidant and cytoprotective properties (Ibitoye *et al*., 2023; Dangani *et al*., 2026). Flavonoids and other polyphenols can directly scavenge free radicals, chelate transition metal ions, and upregulate endogenous antioxidant enzymes, thereby protecting cells from oxidative damage (Intharuksa *et al*., 2024). The hydromethanolic extract of *B. longipedicellata* has demonstrated anti-inflammatory activity through inhibition of COX-2 and TNF-α (Oladoja *et al*., 2024), while additional studies have confirmed its antidiabetic, antioxidant, and hepatoprotective effects in rats (Oladoja *et al.*, 2025).

Despite the ethnopharmacological relevance of *B. longipedicellata* in treating male infertility, no scientific investigation has evaluated its potential to protect against hypothalamic-pituitary toxicity. This study therefore represents the first examination of the plant's therapeutic potential in the context of neuroendocrine reproductive damage. The present investigation was designed to evaluate the protective effects of ethanol leaf extract of *Baphia longipedicellata* on metronidazole-induced oxidative stress and cytoarchitectural alterations in the hypothalamic-pituitary axis of male Wistar rats. Therefore, it was hypothesized that ethanol leaf extract of *Baphia longipedicellata* would attenuate metronidazole-induced oxidative stress and preserve the cytoarchitecture of the hypothalamic–pituitary axis in male Wistar rats.

MATERIALS AND METHODS

Experimental Design and Study Area

Animal Grouping

Metronidazole (MTZ; batch number N85306629) was obtained from Forever Pharmacy Company, Nanjing Xin'gang Development Zone, Qixia District, Nanjing City. The drug was stored according to the manufacturer's instructions and prepared fresh in distilled water immediately before administration. The MTZ dose (500 mg/kg) was selected based on El-Nahas and El-Ashmawy (2004). Twenty-five (25) adult male Wistar rats (190–210 g) were sourced from the Animal House of Olabisi Onabanjo University, Ogun State, Nigeria. They were housed in well-ventilated plastic and wire mesh cages, with five rats per cage, and given a 14-day acclimatization period before the administration of extracts commenced. The rats were provided with feed and clean water ad libitum, and their bedding was changed daily.

Twenty-four hours after the last drug administration, at the end of the experimental period, the animals were euthanized with ketamine (40 mg/kg i.p.) and xylazine (4 mg/kg i.p.). The whole brain (hypothalamus and pituitary gland) was immediately extracted, weighed, and kept in appropriate medium for subsequent bioassays and histological analysis (Akano *et al*., 2026). For biochemical assays, the hypothalamic-pituitary tissue was homogenized in 1:5 freshly prepared 0.1 M Phosphate buffer saline (pH 7.4) and the homogenate was stored at -80°C for the bioassays.

To evaluate the ameliorative potential of ethanol extract of *Baphia longipedicellata*, hypothalamic-pituitary oxidative stress was induced in this study via a 14-day administration of metronidazole.

Table 1: Experimental Animal Grouping

Group	Treatments	Duration of Administration	Route	References
A: Control	10ml/kg Distilled water	68days	Oral gavage	
B: MTZ only	500mg/kg MTZ	14 days	Oral gavage	(El-Nahas and El-Ashmawy, 2004)
C: MTZ + ELEBL(200mg)	500mg/kg MTZ + 200mg/kg of EEBL	14 + 54 days	Oral gavage	(Oladoja *et al*., 2024)
D: MTZ + ELEBL(400mg)	500mg/kg MTZ + 400mg/kg of EEBL	14 + 54 days	Oral gavage	(Oladoja *et al*., 2024)
E: MTZ + withdrawal period	500mg/kg MTZ	14 + 54 days	Oral gavage	(El-Nahas and El-Ashmawy, 2004)

MTZ indicates Metronidazole, ELEBL indicates ethanol leaf extract of Baphia longipedicellata.

Collection and Preparation of Ethanol Extract of *Baphia longipedicellata* Leaf

Fresh leaves of *Baphia longipedicellata* were procured from Ibadan around the neighborhood of the Federal Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State. The identity of the plant was confirmed by Prof. S. A. Odewo, and the leaf was assigned voucher number FHI1135620 at FRIN. The leaves were cleaned, air-dried for 28 days and then ground into powdered form.

The ethanolic extraction of the plant leaf material was conducted following the protocols established by Fatmawati *et al*. (2019) and Chukwudi and Ezeabara (2018). A 500 g powdered leaf sample was macerated in 2.5 L of ethanol (1:5 w/v) at room temperature for 72 hours. The mixture was subsequently filtered and the resultant filtrate was concentrated using a rotary evaporator at 40°C. The final extract was stored in airtight containers under refrigeration for subsequent analyses.

Ethical Consideration

Ethical approval was obtained at OUTHREC, Olabisi Onabanjo University Teaching Hospital, Health Research and Ethics Committee with approval details, ref: ID OOUTH/HREC/02-2026/081/E564/2026AP, and the experimental procedures conducted in this study followed the Institutional Animal Care and Use Committee (IACUC) guidelines.

Biochemical Assays

Assessment of Hypothalamic-Pituitary Antioxidant Enzymes and Oxidative Stress Markers

Antioxidant defense markers including hypothalamic-pituitary tissue homogenate levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) were determined using validated colorimetric methods as described by Ajayi *et al*. (2023) and Hamed *et al*. (2025).

Histological Examination

Hypothalamic-pituitary tissues were harvested and immediately fixed in 10% buffered formal saline for at least 24–48 hours to preserve tissue architecture. Following fixation, the tissues were processed using standard histological techniques, involving sequential dehydration in ascending grades of ethanol, clearing in xylene, and infiltration with molten paraffin wax. The processed tissues were then embedded in paraffin blocks, and serial sections of 5 µm thickness were cut using a rotary microtome. The sections were mounted onto clean glass slides and allowed to adhere before being subjected to Hematoxylin and Eosin (H&E) and Cresyl Fast Violet staining.

Staining involved immersion in hematoxylin to stain cell nuclei, followed by differentiation, bluing, and counterstaining with eosin to highlight cytoplasmic and extracellular matrix components. After staining, the slides were dehydrated, cleared, and mounted with coverslips using a resinous mounting medium.

The stained sections were examined under a light microscope at ×400 magnification to assess histoarchitectural changes in the hypothalamus and pituitary gland, in accordance with the photomicrographs presented in Plates 1 - 3.

Cresyl Fast Violet (CFV) staining was carried out to demonstrate Nissl substances and assess neuronal morphology within the tissue sections. Following tissue processing, paraffin-embedded tissue sections of 3–5 µm thickness were mounted on clean glass slides and subjected to deparaffinization and rehydration. The sections were immersed in xylene for 15 minutes to remove paraffin wax, followed by rehydration through descending grades of ethanol (100%, 90%, 80%, and 70%) for 5 minutes each, and then rinsed twice in distilled water. Descending ethanol grades were used because xylene is miscible with absolute ethanol but not with water; the gradual replacement of xylene by water prevents abrupt osmotic changes that could cause tissue shrinkage, swelling, distortion, or detachment and it ensures uniform penetration of the aqueous Cresyl Fast Violet stain.

The tissue sections were stained with Cresyl Fast Violet solution for 10–15 minutes to demonstrate Nissl granules within neuronal cell bodies. Following staining, the sections were rinsed briefly in distilled water and differentiated in 95% ethanol containing a few drops of acetic acid until optimal staining intensity was achieved.

The sections were subsequently dehydrated through ascending grades of ethanol, cleared in xylene, and mounted with DPX mounting medium under a coverslip for microscopic examination. Neuronal cell bodies containing Nissl substances appeared purple to violet against a lighter background.

Parameters assessed included neuronal integrity, distribution of Nissl substance, presence of vacuolations, degeneration of acidophils and basophils in the pituitary gland, and overall cellular architecture.

Statistical Analysis

The data collected in this study were analyzed using GraphPad Prism (version 10.0) and the Statistical Package for the Social Sciences (SPSS) software (version 25.0). Data were analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test for multiple pairwise comparisons. Results are presented as mean ± standard error of the mean (SEM), with n=5 rats per group. Statistical significance was set at $p < 0.05$ for all comparisons.

RESULTS AND DISCUSSION

Effects of ELEBL on Hypothalamic-Pituitary Superoxide Dismutase (SOD) Activity

Metronidazole administration significantly reduced hypothalamic-pituitary SOD activity relative to the control group ($p < 0.0001$) (Plate 1a). The MTZ-only group showed a marked decline in enzyme activity, indicating depletion due to oxidative stress. Both doses of ELEBL (200 mg/kg: $p = 0.0012$; 400 mg/kg: $p < 0.0001$) significantly improved enzyme activity compared with the MTZ-only group. Rats in the withdrawal group continued to show significantly reduced SOD activity ($p = 0.0004$ vs control).

The depletion of SOD activity by MTZ reflects the overwhelming generation of superoxide radicals that exceed the enzyme's catalytic capacity, leading to its inactivation and degradation (Anwar *et al*., 2025). Superoxide dismutase constitutes the first line of enzymatic defense against ROS, catalyzing the dismutation of superoxide anions into hydrogen peroxide and molecular oxygen (Anwar *et al*., 2025). The restoration of SOD activity by ELEBL can be attributed to the direct scavenging of superoxide radicals by flavonoids and polyphenolic compounds present in the extract, thereby reducing the burden on the enzyme and preserving its activity (Intharuksa *et al.*, 2024). Additionally, the extract may upregulate the expression of SOD genes through activation of the Nrf2/ARE signaling pathway, a mechanism that has been documented for several plant-derived antioxidants (Oladoja *et al*., 2025).

Effects of ELEBL on Hypothalamic-Pituitary Catalase (CAT) Activity

MTZ significantly lowered hypothalamic-pituitary CAT activity compared with controls ($p < 0.0001$) (Plate 1b). Both ELEBL doses significantly increased CAT activity relative to the MTZ-only group (200 mg/kg: $p = 0.0021$; 400 mg/kg: $p < 0.0001$), with the 400 mg/kg dose yielding values close to those of the control group ($p = 0.0892$ vs control, ns). The withdrawal group maintained significantly reduced CAT activity ($p = 0.0006$ vs control).

Catalase plays a critical role in detoxifying hydrogen peroxide, converting it to water and oxygen, and its depletion leads to the accumulation of this reactive species, which can diffuse across cell membranes and generate hydroxyl radicals through Fenton chemistry (Rasheed, 2024). The observed reduction in CAT activity following MTZ administration is consistent with the findings of Vyas *et al*. (2022), in which similar depletion in testicular tissue was reported. The restoration of CAT activity by ELEBL suggests that the extract not only scavenges ROS directly but also protects the enzyme from oxidative inactivation and may stimulate its

synthesis (Liu *et al*., 2023). The superior effect of the 400 mg/kg dose indicates a dose-dependent protective mechanism.

Effects of ELEBL on Hypothalamic-Pituitary Glutathione (GSH) Level

MTZ treatment caused a significant depletion of hypothalamic-pituitary GSH compared with the control group ($p < 0.0001$) (Plate 1c). Both ELEBL doses significantly elevated GSH concentrations relative to the MTZ-only group (200 mg/kg: $p = 0.0004$; 400 mg/kg: $p < 0.0001$). The withdrawal group continued to show significantly lower GSH levels than controls (p=0.0012 vs control).

Glutathione is the most abundant non-enzymatic antioxidant in the central nervous system, functioning as a cofactor for glutathione peroxidase and glutathione S-transferase, and directly neutralizing ROS through its sulfhydryl group (Knudsen *et al*., 2026). The depletion of GSH by MTZ reflects its consumption during the detoxification of ROS and nitro radical anions generated by the drug (Chen *et al*., 2024). The restoration of GSH levels by ELEBL may involve multiple mechanisms, including the provision of cysteine precursors for GSH synthesis, upregulation of γ-glutamylcysteine synthetase, and direct protection of GSH from oxidation by the extract's polyphenolic compounds (Oladoja *et al*., 2025).

Effects of ELEBL on Hypothalamic-Pituitary Malondialdehyde (MDA) Level

The MTZ-only group showed a significant rise in hypothalamic-pituitary MDA compared with controls ($p < 0.0001$), indicating increased lipid peroxidation (Plate 1d). Both ELEBL treatments significantly attenuated the MTZ-induced increase in MDA (200 mg/kg: $p = 0.0008$; 400 mg/kg: $p < 0.0001$). The 400 mg/kg dose demonstrated superior reduction of MDA levels, with values comparable to the control group. The withdrawal group maintained elevated MDA levels.

Malondialdehyde is a stable end-product of lipid peroxidation and serves as a reliable biomarker of oxidative damage to cellular membranes (Cordiano *et al*., 2023). The hypothalamic-pituitary region is particularly rich in polyunsaturated fatty acids, making it vulnerable to lipid peroxidation, which compromises membrane fluidity, ion channel function, and receptor integrity (Yang *et al*., 2025). The elevation of MDA by MTZ indicates significant oxidative damage to neuronal and endocrine cell membranes, which can impair signal transduction and hormone secretion. The reduction of MDA levels by ELEBL confirms the extract's ability to inhibit lipid peroxidation, likely through direct scavenging of lipid peroxyl radicals and protection of membrane polyunsaturated fatty acids (Intharuksa *et al*., 2024)

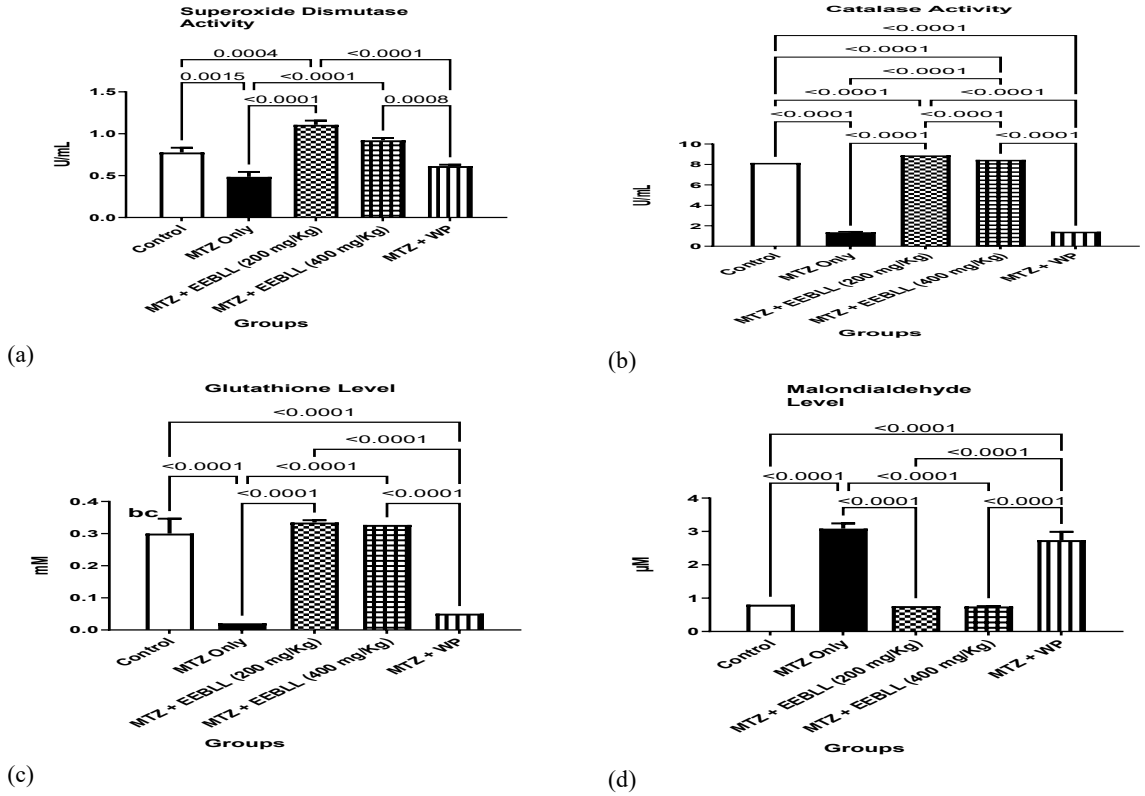


Plate 1: Effect of ethanol leaf extract of *Baphia longipedicellata* on hypothalamic-pituitary oxidative stress markers (A. SOD; B. CAT; C. GSH; D. MDA) of male Wistar rats exposed to MTZ. Values are expressed as mean ± SEM (n=5). Statistical significance at $p<0.05$ using Tukey's Post-Hoc test

KEY:

Group A (Control): Received 10mL/kg of water for 68 days.

Group B (MTZ ONLY): Received 500 mg/kg of metronidazole (MTZ) for 14 days

Group C [(MTZ + EEBLL (200mg)]: Received 500 mg/kg of MTZ for 14 days followed by 200 mg/kg of ethanol leaf extract of *Baphia longipedicellata* for the next 54 days.

Group D [(MTZ + EEBLL (400mg)]: Received 500 mg/kg of MTZ for 14 days followed by 400 mg/kg of ethanol extract of *Baphia longipedicellata* leaf for the next 54 days.

Group E (MTZ + WP): Received 500 mg/kg of MTZ for 14 days, followed by a 54-day withdrawal period.

Effects of ELEBL on Hypothalamic Histoarchitecture

Hematoxylin and Eosin (H&E) staining of the hypothalamus revealed that the control group (Group A) showed normal hypothalamic architecture characterised by intact neurons with well-defined cell bodies, prominent nuclei, and minimal intercellular spaces. In contrast, exposure to MTZ only (Group B) induced marked histopathological alterations, including widespread neuronal degeneration, cytoplasmic vacuolation, ischemic changes, and pyknosis. MTZ+200mg ELEBL (Group C) resulted in moderate improvement, with reduced vacuolation (blue arrow) and neuronal degeneration, although mild inflammatory cell infiltration and residual vacuoles persisted (yellow arrow). MTZ +400mg ELEBL (Group D) maintained normal cytoarchitecture comparable to the control. Recovery group (Group E) offered limited protection, showing persistent neuronal degeneration, vacuolation, and gliosis similar to group B (Plate 2).

The neuronal degeneration observed in the MTZ-only group is consistent with the findings of Ajibade and Ayanlade (2021), in which conspicuous degeneration of the cerebral cortex was reported following MTZ administration. The mechanism underlying this neuronal damage involves the generation of ROS that attack neuronal membranes, mitochondria, and DNA, triggering apoptotic and necrotic cell death (Chen *et al*., 2025). Vacuolation reflects intracellular edema resulting from membrane pump dysfunction, while pyknosis indicates irreversible chromatin condensation associated with apoptosis (Pasternak *et al*., 2026). The hypothalamus is particularly vulnerable to oxidative injury because of its high density of mitochondria and the presence of specialized neurons that regulate GnRH secretion (Roepke & Sadlier, 2021).

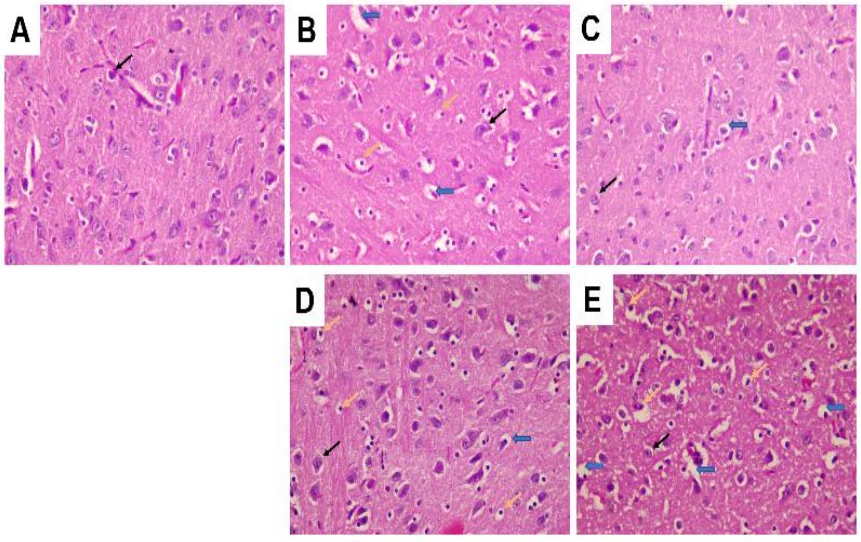


Plate 2: Photomicrographs of the hypothalamus stained with H&E at (X400). Black arrow indicates normal neurons, blue arrow=Vacuolations, yellow arrow=Pyknosis/ degenerated neurons. Group A is the control group, B is the MTZ only, C =MTZ+200mg ELEBL, D= MTZ +400mg ELEBL and E= MTZ+Recovery

Group A neuronal architecture is largely preserved. Cells appear well-defined with intact nuclei and well distributed Nissl substance indicative of healthy neurons. Group B revealed reduced number of clearly identifiable neurons with faint or fragmented Nissl substance. Marked glial proliferation with reduced neuronal clarity and increased cellular crowding indicating a moderate neuronal degeneration characterized by progressive chromatolysis and impaired protein synthesis. Group C shows an increased number of neurons with numerous small, darkly stained cells (glial cells). Group D demonstrates a more pronounced neuroprotective effect, with the Nissl features closely resembling those of the control group. Group E displays marked depletion of Nissl-positive neurons, marked glial cell proliferation, extensive vacuolation and structural disorganization indicative of impaired protein synthesis and severe neuronal loss (Plate 3).

The preservation of Nissl substance in ELEBL-treated groups is significant because Nissl bodies are essential for the synthesis of proteins, including neurotransmitters and their receptors that are required for normal hypothalamic function (Bhati *et al*., 2023). The loss of Nissl substance in the MTZ-only group indicates severe impairment of neuronal protein synthesis, which would compromise the synthesis and pulsatile release of GnRH. The restoration of Nissl substance by ELEBL suggests that the extract preserves the biosynthetic machinery of hypothalamic neurons, thereby maintaining their capacity to regulate pituitary function (Bhati *et al*., 2023).

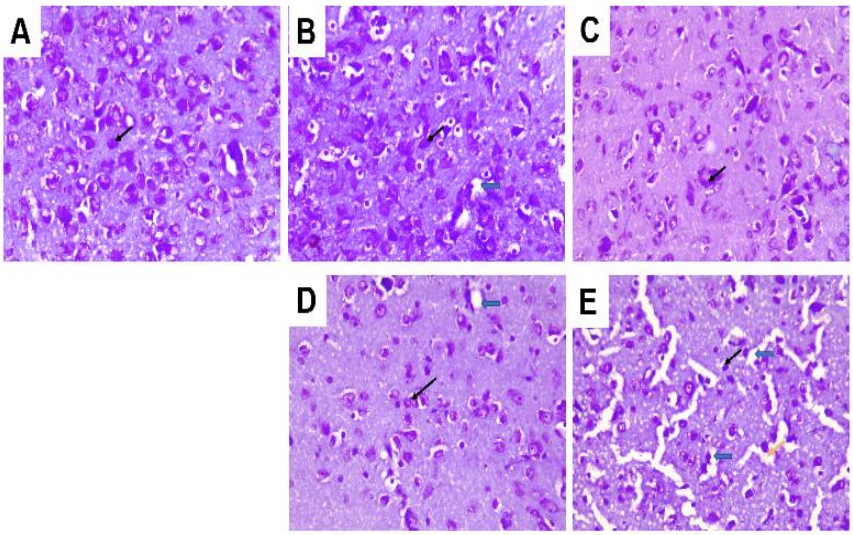


Plate 3: Photomicrographs of sections of the hypothalamus stained with Cresyl Fast Violet. X400. Black arrow indicates normal neurons, blue arrow=Vacuolations, yellow arrow=Pyknosis/ degenerated neurons. Group A is the control group, B is the MTZ only, C =MTZ+200mg ELEBL, D= MTZ +400mg ELEBL and E= MTZ+Recovery

Effects of ELEBL on Pituitary Histoarchitecture

Cresyl Fast Violet staining confirmed these findings, with the Control group shows intact structural architecture of acidopils and basophils. Group B showing severe degeneration of acidopils and basophils. Group C and D showing numerous and conspicuous cells. Group E showing mild degeneration of acidopils and basophils compared with the control (Plate 4 and Plate 5).

The pituitary gland is composed of three principal cell types: acidophils (somatotrophs and lactotrophs), basophils (gonadotrophs, thyrotrophs, and corticotrophs), and chromophobes (Anapathy & Tadi, 2023). The gonadotrophs, which are basophilic, are responsible for the synthesis and secretion of LH and FSH, hormones that are essential for testicular function and spermatogenesis (Clavijo & Hsiao, 2018). The severe degeneration of acidophils and basophils in the MTZ-only group indicates that MTZ induces oxidative damage to pituitary cells, compromising their structural and functional integrity (Ogbonye *et al*., 2020). This degeneration would impair the production and secretion of gonadotropins, leading to downstream testicular dysfunction and impaired fertility (Vyas *et al*., 2022).

The preservation of acidophils and basophils in ELEBL-treated groups demonstrates the cytoprotective effects of the extract on pituitary cells. The extract's antioxidant properties likely prevent the oxidative damage that would otherwise lead to cellular degeneration and compromise gonadotropin secretion (Oladoja *et al*., 2025). The presence of numerous and conspicuous cells in the ELEBL-treated groups suggests that the extract not only protects existing cells but may also promote cellular regeneration and proliferation (Intharuksa *et al*., 2024). The preservation of pituitary architecture is critical for maintaining the hormonal cascade that regulates testicular function.

Prior research has documented its anti-inflammatory, antidiabetic and hepatoprotective activities, but this is the first study to demonstrate its protective effects on the hypothalamic-pituitary axis against oxidative stress. These findings, together with its antioxidant properties, broaden the therapeutic potential of this plant and reinforce its traditional use as a remedy for male infertility and related conditions. The study suggests that *B. longipedicellata* may represent a novel phytotherapeutic option for the prevention and management of reproductive complications associated with oxidative stress-inducing conditions.

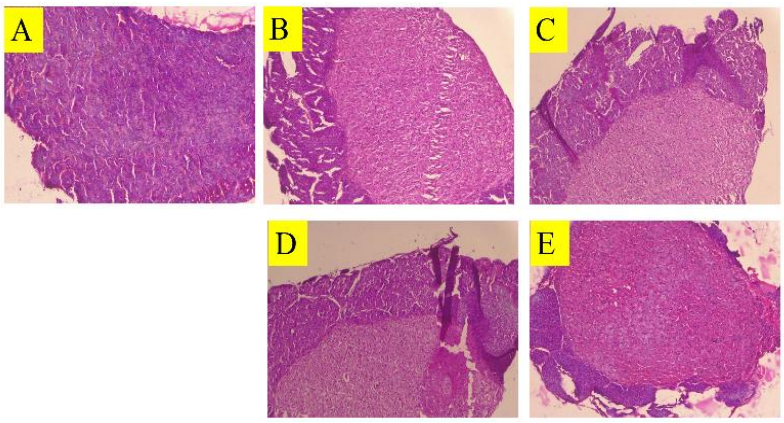


Plate 4: Photomicrographs of the Pituitary stained with H&E at (X400). Group A is the control group, B is the MTZ only, C =MTZ+200mg ELEBL, D= MTZ +400mg ELEBL and E= MTZ+Recovery

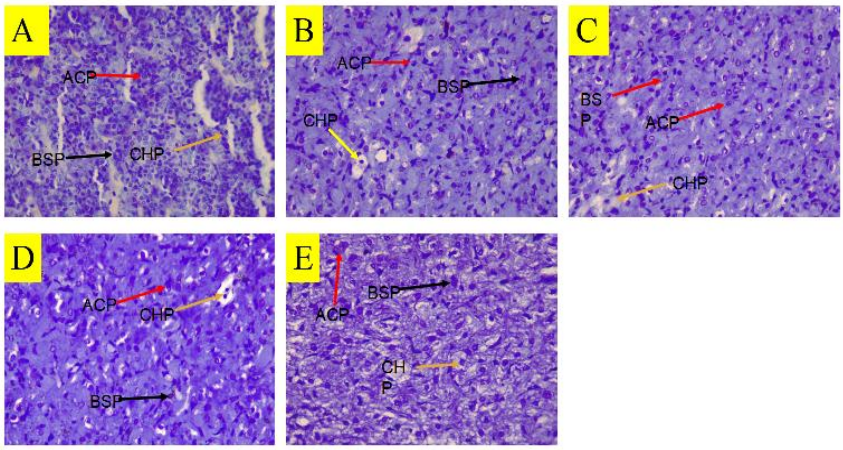


Plate 5: Photomicrographs of sections of the pituitary gland stained with Cresyl Fast Violet. X400. Group A is the control group, B is the MTZ only, C =MTZ+200mg ELEBL, D= MTZ +400mg ELEBL and E= MTZ+Recovery. Red arrow indicates acidopils, Black arrow = basophils, Yellow arrow = Chromophobes

CONCLUSION

The ethanol leaf extract of *Baphia longipedicellata* effectively attenuates metronidazole-induced oxidative stress in the hypothalamic-pituitary axis by restoring antioxidant enzyme activities (SOD, CAT, GSH) and reducing lipid peroxidation (MDA). The extract also preserves hypothalamic-pituitary cytoarchitecture, preventing neuronal degeneration in the hypothalamus and degeneration of acidophils and basophils in the pituitary gland. The 400 mg/kg dose showed superior protective effects compared to the 200 mg/kg dose.

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